

## Background

Formalin test is a widely used method to measure analgesic effect. Injection of formalin into one hindpaw of rats elicits behavioural signs of pain, such as licking of the paw. Our goal was to replace the traditional and time consuming observational work of humans by an automated method. A special new algorithm was designed for LABORAS™, an automated system to measure and analyse rodent behaviour, allowing parallel observation of 4-8 rats. First, we compared the LABORAS™ measurement and observers' scores and then we determined ED<sub>50</sub> of several well-known compounds.

## Methods

Rats were pretreated orally just before formalin injection with four different treatment (at least 3 animals in each group). After pretreatment all rats were injected with 50 µl 2.5% formalin in the right hind limb dorsally and observed by the LABORAS™ equipment and, at the same time, recorded on video from the side of cage for at least 30 minutes following the injection. To improve the visibility of rat when facing away from the camera, two mirrors were placed beside and behind the cage. The duration of experiments was between 30 and 60 minutes.

For pharmacological validation we measured the time spent with licking after the administration of different compounds with known analgesic effect belonging to different classes regarding their mechanism of action.

Formalin injection into hindpaw produces a characteristic biphasic curve of pain related licking behavior. First phase (0-10 min) represent acute pain, while second phase (15-25 min) reflects peripheral activity.

## Statistical analysis

Data are presented as means ± SEM. Statistical comparisons between mean of observers and LABORAS™ data were performed using unpaired t-test at each interval (Instat, GraphPad, San Diego, USA). Significance was considered at 0.05 level (\* indicates p<0.05).

In pharmacological validation analysis of variance (ANOVA) with post hoc Tukey-test (Instat, GraphPad, San Diego, USA) was used to compare the effect of different drugs between the groups.

Asterisks (\*, \*\* and \*\*\*) indicate p<0.05, p<0.01 and p<0.001, respectively.

## Results II. Pharmacological validation

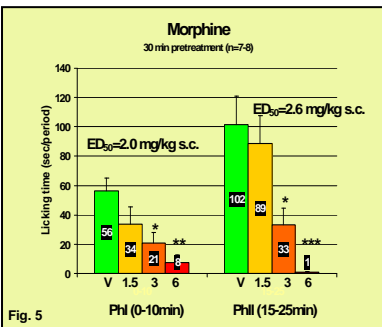


Fig. 5

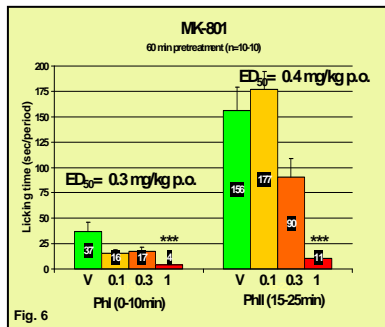


Fig. 6

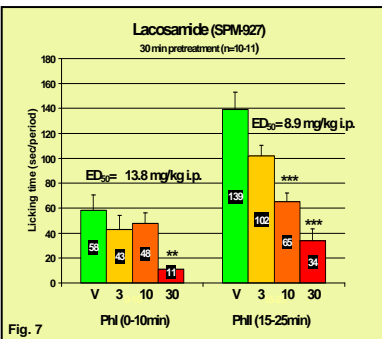


Fig. 7

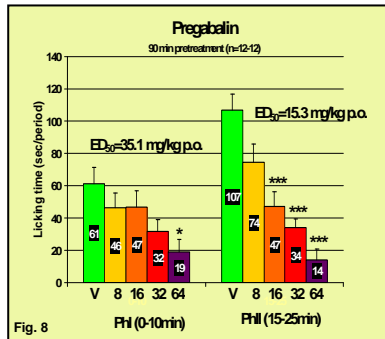


Fig. 8

## Conclusion

No significant difference could be observed between LABORAS™ new formalin software and observers' scores. Furthermore, we measured different compounds and calculated ED<sub>50</sub> in 1st and 2nd phase in rat formalin test. The calculated ED<sub>50</sub> values measured with LABORAS formalin test are in agreement with previous findings.

Our conclusion is that LABORAS™ new formalin software provides a fast and reliable measurement to assess the effects of analgesic compounds.

## Results I. Comparison between scores

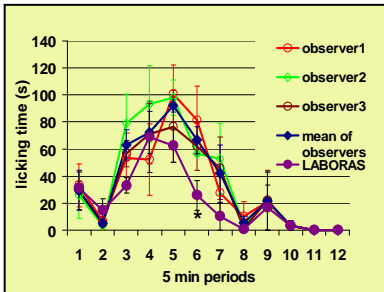


Fig. 1 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with saline p.o. (n=3-3). \*p<0.05 t-test LABORAS vs mean of observers

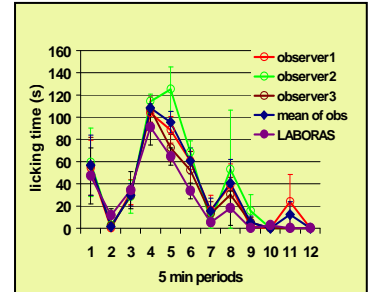


Fig. 2 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with vehicle p.o. (n=3-3).

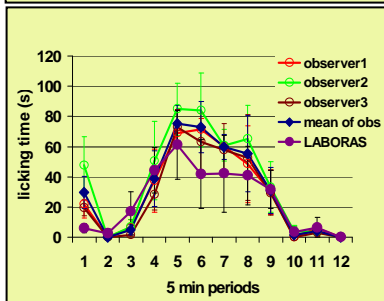


Fig. 3 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with ineffective RG compound p.o. (n=3-3).

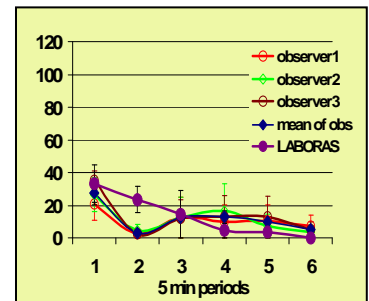


Fig. 4 Comparison between LABORAS™ formalin test and observers' scores after pretreatment with 1 mg/kg MK-801 p.o. (n=3-3).

## Results I.

Individual experiments were evaluated by LABORAS™ and three observers. The analysis of LABORAS™ and observers' scores were compared. After pretreatment with saline no significant difference could be observed between Laboras data and mean of observers' scores at each intervals except 6th interval where the difference reached the significance level (Fig. 1).

In the measurement of vehicle, RG compound or MK-801 no significant difference could be determined at all between LABORAS™ data and mean of observers' scores

(Fig. 2, 3 and 4).

## Results II.

For pharmacological validation we measured the time spent with licking after the administration of different doses of compounds. We determined ED<sub>50</sub> in the 1<sup>st</sup> and 2<sup>nd</sup> phase (mg/kg) of the opioid agonist morphine s.c. (Fig. 5), the channel blocker type NMDA antagonist MK-801 p.o. (Fig. 6), a glycine site NMDA antagonist lacosamide i.p. (SPM-927) (Fig. 7), and a calcium channel antagonist pregabalin p.o. (Fig. 8).

The results of LABORAS™ formalin test were in accordance with previous findings.

## Discussion

Evaluating the LABORAS™ formalin result and observers' scores data we can find that the difference between observers and Laboras is not higher than it can be measured between observers alone. The highest difference can be found at the descending part of the 2<sup>nd</sup> phase. This is the part of pain related licking behavior where animals are licking their paw frequently for short time.

There is a possibility of a small systemic error made by LABORAS™ or by observers, as well. Probably, the longer reaction time of humans can make such difference. The difference is small and seems not to disturb reliable assessment of inhibition by a drug which was the main goal of the test.



Figure 9. LABORAS™ triangle-shaped platform with the home-cage system.